



The University of Alabama at Birmingham  
Comprehensive Cancer Center  
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October 17, 1985

NASA/MSFC  
Attn: JA63/W. W. Moore, Jr.  
Marshall Space Flight Center, Alabama 35812

Dear Wally:

I am writing in regards to NASA Contract #NAS8-36611, "Protein Crystal Growth in a Microgravity Environment", for which I am Principal Investigator. Enclosed is the final report describing our experimental results from shuttle flight 51-F.

If agreeable with you, regular monthly reports will be submitted at the end of each month, starting with the end of October, 1985.

Thank you very much for your kind assistance.

Sincerely,

Charles E. Bugg  
Associate Director, U.A.B.  
Comprehensive Cancer Center

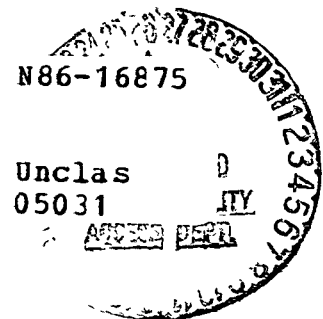
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cc: Mr. Glen Daniel Oldre  
Contract Administrator

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RESULTS FROM SHUTTLE FLIGHT 51-F Final  
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Flight 51-F"

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Prepared for: George C. Marshall Space Flight Center,  
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## PROTEIN CRYSTAL GROWTH RESULTS FROM SHUTTLE FLIGHT 51-F

We have now completed the initial analysis of the PCG experiments that were run on 51-F.

### Experimental Procedures

- (1) We flew the same general hand-held unit that had been flown on 51-D. Several major modifications had been made in the hardware:
  - (a) We added plungers to close the sample syringes during launch and landing, since we had lost samples from our syringes at those two stages on 51-D.
  - (b) We replaced the plastic syringe tips with ones made of glass. Most of the experiments involved cylindrical glass tips with blunt ends, and these tips were tested on a KC-135 flight.
  - (c) We included six glass syringe tips that were bell-shaped; the protein solutions were held within the bells during flight.
  - (d) Four of the protein drops were tethered during flight, by using the opposing plungers as tether points.
  - (e) Teflon tape was placed around eight of the glass syringe tips, to determine if this hydrophobic barrier would enhance drop stability.
- (2) We flew four different proteins on 51-F: lysozyme,  $\alpha 2$  interferon, bacterial purine nucleoside phosphorylase (PNP), and human C-reactive protein (CRP). These four proteins were selected because:
  - (a) We have considerable experience crystallizing these particular proteins at U.A.B..
  - (b) They permitted us to assess drop stability for solutions that contained different types of precipitating agents. Bacterial PNP is crystallized from ammonium sulfate salt solutions; C-reactive protein is crystallized from methylpentanediol; lysozyme is crystallized from NaCl solutions; and  $\alpha 2$  interferon is crystallized from polyethylene glycol. Lysozyme usually crystallizes easily, so it serves as a useful control. The other three proteins generally crystallize within two days, but crystals of these proteins obtained in our laboratory at U.A.B. are small, and diffract only to moderate resolutions.
- (3) The PCG experiments on 51-F were performed by Drs. Loren Acton and Story Musgrave. Larry DeLucas and I met with these two crew members for about two hours prior to 51-F, to instruct them on the experimental procedures that were involved.
- (4) Our experiments included a dialysis block, and two individual syringe assemblies. Loren Acton assumed responsibility for activating the dialysis experiment, and for activating and later deactivating one of the syringe

assemblies. Story Musgrave assumed responsibility for activating and deactivating the second syringe assembly.

- (5) The dialysis unit contained six different dialysis cells. Lysozyme was the protein that was used for all of these dialysis experiments. The two individual chambers in the dialysis unit contained different concentrations of sodium chloride, and each chamber contained three of the dialysis cells.
- (6) Each of the two syringe assemblies contained seventeen chambers. All of the experiments within these chambers were of the vapor diffusion type. The reservoir solutions for these experiments were held by the same cotton wicking material that was used on 51-D. However, unlike 51-D, the reservoir solutions were added to the wicks prior to launch and were separated from the protein syringes by a metal grid.
- (7) Loren Acton began activating the dialysis experiments and one of the syringe systems one hour and 25 minutes into the mission. This unit was activated as early as possible, so that we could assess the effects of OMS burns on drop stability. The syringes were activated by Dr. Acton holding the assembly on his lap while he was strapped in a flight seat.
- (8) The second syringe system was activated by Story Musgrave thirty-six hours into the mission. These syringes were activated while the syringe assembly was mounted securely above a light box. Most of the OMS burns had been completed before this second unit was activated, but at least one major OMS burn occurred after the unit was activated.
- (9) The syringe units were deactivated during the seventh day of the mission.
- (10) Photographs were taken of the syringe units after activation and before deactivation. Unfortunately, these photographs did not show much detail, since each frame depicts a complete syringe assembly (17 syringes).
- (11) The protein sample volumes ranged from 25  $\mu$ ls to 80  $\mu$ ls for the syringe experiments, and 50  $\mu$ ls of protein solution was included in each of the dialysis cells. The reservoirs for the vapor diffusion experiments contained 500-650  $\mu$ ls. The dialysis chambers each contained about 35 mls of salt solution.
- (12) Larry DeLucas retrieved the hardware in California. The hardware was delivered to Dr. DeLucas within two hours after the shuttle landing. He immediately photographed each chamber and recorded any significant observations while viewing the chambers through a microscope. He arrived back in Birmingham approximately 8 hours after receiving the hardware in California, and proceeded with analysis of the samples at U.A.B.
- (13) Information concerning the course of the experiments aboard the shuttle were obtained from dictated notes by Loren Acton and Story Musgrave, and by later personal interviews with these two crew members.

## Experimental Results

- (1) Activation of experiments by Loren Acton:
  - (a) Dialysis experiments were successfully activated less than 2 hours into the flight.
  - (b) The three bell-shaped tips in Dr. Acton's syringe assembly were successfully filled with solution.
  - (c) The two tethered droplets were successfully formed and stabilized.
  - (d) Four droplets were lost during deployment. None of these droplets were on the four syringe tips that contained teflon tape.
- (2) Activation of experiments by Story Musgrave:
  - (a) The plungers on the three bell-shaped syringe tips were extruded beyond the mouth of the bell, and the protein samples ran behind the plunger back into the syringe barrels.
  - (b) Three of the glass syringe tips rotated with the plungers, so the drops could not be extruded. Two of these rotating syringe tips were among the four tips in the apparatus that were covered with teflon tape.
  - (c) The two tethered drops were successfully deployed and joined with the tethering plunger.
  - (d) The remaining nine droplets were successfully deployed.
- (3) Three droplets were lost from Loren Acton's syringe assembly during flight. It is not known if these drops were lost during OMS burns, or were lost for other reasons.
- (4) One droplet was lost from Story Musgrave's syringe assembly during flight. He observed the apparatus after a major OMS burn, and all drops were still intact.
- (5) Upon deactivating his syringe assembly, Dr. Acton observed crystals in all three of the bell tips, and crystals that were attached to the tips of all seven syringes that retained droplets during flight. According to our instructions, those syringe tips that had attached crystals after the drops were withdrawn were not sealed by the closing plungers prior to re-entry. Those protein crystals that had been observed by Dr. Acton included CRP, lysozyme, interferon and bacterial PNP. Three of the samples that were lost during flight were from syringe tips that contained teflon tape.
- (6) Story Musgrave observed no crystals in his syringe assembly.
- (7) When Dr. DeLucas recovered the hardware at the landing site in California, he noticed that the syringe chambers contained considerable condensation, suggesting that the apparatus had been through a significant temperature fluctuation. Mr. Bill Paton, the NASA employee who recovered the hardware from the shuttle, noted that the temperature in the NASA van had reached 90°F due to the extreme heat on the runway.

- (8) Three of the six dialysis cells had been ruptured, apparently during the activation process. The three surviving cells contained large tetragonal crystals of lysozyme with average dimensions of  $1.3 \times 0.65 \times 0.65$  mm.
- (9) When the hardware was examined at U.A.B., no crystals were observed attached to the tips of the syringes.
- (10) Loren Acton's syringe assembly contained crystals in four of the protein solutions, but all of these crystals were small and were of poor quality. The crystals that were observed at U.A.B. in the solutions from the syringes included lysozyme, interferon, and bacterial PNP. No crystals of CRP were observed.
- (11) Story Musgrave's unit had four syringes that contained crystals when analyzed at U.A.B. These crystals were also small and of poor quality. The crystals from Dr. Musgrave's unit included interferon, lysozyme and bacterial PNP.
- (12) No samples were lost from the syringes upon landing. Four of the syringes in Dr. Acton's assembly had not been closed with plungers prior to landing, since he had observed crystals growing off the tips of the syringes. All four of these samples remained in the syringes during landing, and seemed to have the expected volume of solution when measured at U.A.B.
- (13) The volumes of the protein solutions in Dr. Acton's syringe assembly indicated that these solutions had reached equilibrium with the reservoirs in the syringe chambers. At equilibrium, the volume of the protein droplets would have been decreased by 50%, which was consistent with the final volumes observed in these syringes.
- (14) All of the protein solutions from Dr. Musgrave's syringe assembly contained larger volumes than would be expected if these protein solutions had reached equilibrium with the reservoir solutions. Overall, the volumes of the protein solutions in these assemblies had only decreased by 30-40%.

#### Summary and Conclusions

- (1) Sample stability was much increased over that observed during the experiments on flight 51-D. Only four droplets were lost during deployment, and these were all lost by Dr. Acton during a period when he was not feeling well, and was trying to activate the syringes while holding the assembly on his lap. Only four droplets were lost during flight, despite an unusually large number of OMS burns. Both Dr. Acton and Dr. Musgrave felt that the current syringe systems are adequate for stabilizing the droplets, if the unit is properly anchored during activation, deactivation and photography.
- (2) The dialysis experiments produced lysozyme crystals that were significantly larger than those obtained in our identical ground-based studies, using the flight hardware and procedures. The lysozyme crystals that we obtained during the ground-based studies were all less than 0.7 mm in the longest dimension, whereas the flight crystals had average dimensions of  $1.3 \times 0.65 \times 0.65$  mm.
- (3) Temperature fluctuations apparently caused problems during the crystallization experiments on 51-F. The condensation observed in the chambers upon retrieval of the hardware in California indicated that the samples had reached an elevated

temperature and then had cooled back to room temperature. The information that we have suggests that this temperature rise took place after the shuttle landed. Presumably, the crystals that were observed by Dr. Acton may have been destroyed by this rise in temperature.

- (4) Our ground-based studies indicated that teflon tape would stabilize droplets on these syringe tips. The teflon tape did seem to assist in drop stability during deployment. However, three drops were lost from taped tips during flight.
- (5) It is interesting that samples survived during the re-entry and landing in glass tips that were not stoppered with plungers. We had lost nearly all of the liquid from syringes on shuttle flight 51-D, but we had used polypropylene syringes on that flight. It is possible that the glass syringe tips hold the liquids more tightly by capillary action, and it is possible that some future experiments can be run without the necessity of using a plunger to close the tips of the syringes on launch and landing.
- (6) It is clear that the protein samples had not completely equilibrated with the reservoirs in Dr. Musgrave's syringe assembly, whereas equilibration appears to have been completed in Dr. Acton's assembly. Dr. Musgrave's experiments were activated some 34 hours after those that were initiated by Dr. Acton, so the time for equilibration was somewhat different. From the ground-based studies, we had expected that equilibration should be complete within 2-4 days for all of these vapor-diffusion experiments. Thus it appears that the vapor diffusion rates are somewhat slower under microgravity conditions.
- (7) Drop tethering was highly successful. All four of the tethered drops were stable, even though they contained MPD solutions.
- (8) The main purpose of the PCG experiments on 51-F was to assess the hardware and experimental procedures that are being developed for future flights, when temperature control will be available. However, we also obtained some encouraging results with lysozyme crystal growth. Using micro-dialysis methods, we obtained lysozyme crystals that are considerably larger than ones obtained on the ground, using the identical apparatus and procedures.

#### Plans for Future Experiments

- (1) We are encouraged by the results relating to drop stability on these blunt-end, siliconized glass tips. We feel that teflon coatings around the outside of the tips further enhances drop stability, and we are now investigating shrink wrapped teflon sleeves for further stabilizing the drops. We are also considering using drops with average volumes that are less than the 80  $\mu$ l drops that were flown on 51-F, in order to enhance drop stability and accelerate equilibration rates.
- (2) We are now running ground-based experiments designed to optimize the equilibration rates in our vapor-diffusion chambers. There are three possible changes that are now being tested:
  - (a) We are experimenting with methods for maximizing the surface area of wicking material by running wick around the chamber walls. Our initial

-50% by  
increasing the wicking surface area, and by bringing the wicking materials closer to the protein droplets.

- (b) We are examining alternatives to the metal grid that we have been using to separate the equilibration reservoir from the protein samples. Our current grid decreases the available surface area considerably, and we are experimenting with other mechanisms for restraining the wicking material.
- (c) We are investigating alternate methods for holding the reservoir solutions, including gels, beads, sponges and physical barriers.
- (3) Since it now appears that solutions may be stable within the glass tips, without the necessity of a stopper mechanism, we plan to try additional liquid-liquid diffusion experiments on future flights. We had temporarily abandoned these experiments, since our current hardware configuration did not permit us to extrude samples from two syringes while also stoppering these syringe tips.
- (4) More attention will be given to photographic procedures, including lighting, film type and choice of lenses.
- (5) Thermal waxes will be used to control the temperature of the samples after they are removed from the shuttle.
- (6) A bracket will be designed to hold the syringe assemblies in the mid-deck area. This bracket will permit syringes to be activated and deactivated, and photography to be performed, while keeping the syringe assemblies in a fixed position.
- (7) In the future, syringe tips will be made of precision glass tubing. This should eliminate the problem that Dr. Musgrave encountered with rotating syringe tips during drop deployment.
- (8) We need to improve the procedures that are used for activating the dialysis experiments, since the technique that we used on 51-F resulted in ruptured membranes on three of the dialysis cells. We are now testing alternative mechanisms for activating these experiments on the shuttle.